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Understanding sodium channel function and modulation using atomistic simulations of bacterial channel structures.

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Abstract

Sodium channels are chief proteins involved in electrical signaling in the nervous system, enabling critical functions like heartbeat and brain activity. New high-resolution X-ray structures for bacterial sodium channels have created an opportunity to see how these proteins operate at the molecular level. An important challenge to overcome is establishing relationships between the structures and functions of mammalian and bacterial channels. Bacterial sodium channels are known to exhibit the main structural features of their mammalian counterparts, as well as several key functional characteristics, including selective ion conduction, voltage-dependent gating, pore-based inactivation and modulation by local anesthetic, anti-arrhythmic and anti-epileptic drugs. Simulations have begun to shed light on each of these features in the past few years. Despite deviations in selectivity signatures for bacterial and mammalian channels, simulations have uncovered the nature of the multi-ion conduction mechanism associated with Na⁺ binding to a high field strength site established by charged glutamate side chains. Simulations demonstrated a surprising level of flexibility of the protein, showing that these side chains are active participants in the permeation process. They have also uncovered changes in protein structure, leading to asymmetrical collapses of the activation gate that have been proposed to correspond to inactivated structures. These observations offer the potential to examine the mechanisms of state-dependent drug activity, focusing on pore-blocking and pore-based slow inactivation in bacterial channels, without the complexities of inactivation on multiple timescales seen in eukaryotic channels. Simulations have provided molecular views of the interactions of drugs, consistent with sites predicted in mammalian channels, as well as a wealth of other sites as potential new drug targets. In this chapter we survey the new insights into sodium channel function that have emerged from studies of simpler bacterial channels, which provide an excellent learning platform, and promising avenues for mechanistic discovery and pharmacological development.

Keywords

Sodium channels; Ion permeation; Inactivation; Drug binding; Molecular dynamics simulation

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INTRODUCTION

Since first identified as the main agent for the transmission of electric signals in nervous cells by Hodgkin and Huxley in 1952 [1], voltage-gated sodium (Na_v) channels have been the center of interest of a rapidly increasing numbers of physiological, biophysical and pharmacological studies. Na_v channels are one of the fundamental molecular components of signalling in our nervous systems. They act to depolarize excitable cells through selective transport of Na^+ ions across cell membranes in response to changes in membrane electrical potential. Na_v channel isoforms can be found in almost all electrically active tissues in the body, from neurons to cardiac and skeletal muscles cells [2], and are important in physiological processes such as regulation of heartbeat, pain sensation, transmission of nervous signals and enablement of cognitive processes [3]. Ion channels are a focus in the development of therapeutic agents [4, 5], representing 13% of all known drugs [6], with particular interest in Na_v channels, being targets for epilepsy, cardiac arrhythmias, chronic and neuropathic pain, migraine, spasticity, tremor, neurodegenerative disease, bipolar disorder, anxiety, obsessive-compulsive disorder, myotonia, dystonia, schizophrenia, restless legs syndrome, post-traumatic stress syndrome, alcohol dependence and withdrawal [7, 8].

Research into Na_v channels entered a new era at the beginning of the 2000s with the discovery of the first bacterial Na_v channel, NaChBac [9]. Unlike its mammalian counterparts, where 4 similar domains are linked to form a long complex single unit, NaChBac presents a simpler structure, reminiscent of potassium channels, with 4 identical and independent subunits (Fig.1) arranged symmetrically to shape a pore across the membrane (Fig.2). The discovery of this first channel was soon followed by many other close relatives [10, 11], all displaying the same structural features [12]. Their simple structure, along with the added practicality of working with prokaryotic material, allowed for rapid progresses in crystallization and determination of their high-resolution X-ray structures, still unavailable for mammalian channels (whose only structure available to date was obtained by low resolution cryo-electron microscopy [13], showing a central pore surrounded by 4 peripheral regions and giving limited insight into their structure/function relationship).

Since the publication of the first X-ray structure of the bacterial Na_v Ab channel in 2011 [14], several other channels have been solved [15–19]. This bacterial family of channels, Bac Na_v , are both structurally and functionally simpler than their human counterparts while sharing essential features of selective ion conduction, voltage activation, inactivation and drug modulation [18, 20–23]. This makes them very attractive to molecular-level studies, and explains the recent emergence of several computational studies [24–32]. In this chapter we explore recent computational investigations that have used bacterial channel structures to begin to explore function and modulation of sodium channels.

The ability of an ion channel to choose which ions it conducts is essential to the generation of electrical signals. Yet the way in which Na_v channels selectively conduct Na^+ over physiologically abundant K^+ , Ca^{2+} and other ions remains an open question. Na_v channels possess an extracellular selectivity filter that is known to control ion discrimination (Fig.2). Na_v channels present glutamate (Glu/E) or aspartate (Asp/D) side chains, thought to create a

high field strength site (S_{HFS}) that preferentially stabilizes the smaller Na^+ over K^+ [14]. However, selectivity is not as simple as a thermodynamic preference at one site, and emerges from subtle differences in multiple-ion conduction mechanisms [33, 34]. These mechanisms are not well defined, and we have not yet pinpointed what is the critical chemical or physical property that makes a protein pore Na^+ -selective. The $BacNa_v$ selectivity signature consists of a tetrameric Glu ring, unlike human channels that exhibit a DEKA (Asp-Glu-Lys-Ala in domains I-IV) or related locus of residues [35] (see Figs.1&3). While this may suggest different strategies exist to enforce Na^+ selectivity [32], investigations are beginning to comprehend the underlying processes responsible for selective conduction across the phylogenetic tree.

Na_v channels are responsible for the rising phase of action potentials. When the neuronal membrane reaches a threshold potential, these channels undergo a conformational change within a few 100 μ s, causing channel opening (gating). In this process, charged S4 segments in the voltage sensing domain (residues R1–4 in Fig.2) move due to the voltage change, pulling on the S4–S5 linker that opens a gate in the pore domain [36]. After some time (ms or longer), ion flow terminates, due to other changes that lead to a non-conducting/inactive structure. Na_v channels rapidly cycle through closed, open and inactivated states, allowing neurons to fire high-frequency trains of action potentials for brain function [37]. Drugs may act via blocking the pore directly, or by stabilising a specific state of the channel. As many drugs target the inactivated states of the channel [38], understanding the molecular events associated with inactivation is important for future pharmacological developments. Functionally, inactivation occurs on two timescales, with fast inactivation resulting from channel occlusion by a domain linker [39] on a timescale comparable to channel opening, and slow inactivation, due to rearrangements in the pore domain [40, 41] on a time course of milliseconds and longer [37]. Slow inactivation plays an important role in drug activity [42, 43] with successful new antiepileptic drugs acting via that mechanism [44]. Bacterial channels lack the linker responsible for fast inactivation, allowing one to focus on slow inactivation, as done with K channels [45].

Na_v inhibitor drugs have the remarkable ability to target specific cells to control sensory or motor functions, without threatening vital processes. This selectivity allows for uses as local anesthetic (LA), antiepileptic drugs (AED), analgesic and anti-arrhythmic agents. Understanding drug modulation has been hampered by conflicting models that have postulated different active sites, pathways and target states. In the 1970's, Hille proposed separate lipophilic and aqueous pathways for drug binding [46] with the more hydrophobic drugs acting via the former, and charged or hydrophilic drugs, such as titratable amines and quaternary ammonium derivatives, via the latter. Charged drugs are thought to block the open channel, whereas neutral drugs bind and dissociate even if the pore is nonconducting, apparently via lipid-bound fenestrations [3, 14, 47–49]. Evidence suggests that therapeutic drugs inhibit via a modulated receptor mechanism, binding more strongly to inactivated channels [50], thereby reducing high-frequency firing. Despite historical focus on fast inactivation [51] it is known that slow inactivation is affected by drugs [42, 52] and that mutation of key residues impairs slow inactivation [53]. Structurally-defined Na_vBac channels (which only slow-inactivate) exhibit drug inhibition like human channels [18, 21, 54], making them ideal targets for drug discovery.

Molecular dynamics (MD) simulation is an essential tool for studying biological systems at the molecular-level [55]. In the last decade, dramatic increases in computer power have led to bigger and longer simulations than ever before (e.g. [56, 57]), allowing studies of an ever-widening array of biological phenomena. The availability of Na_vBac X-ray structures has enabled the use of atomic-level simulations to further understand function. In addition, modern computational architectures have enabled simulations of realistic models of membrane ion channel systems on the multi- μ s timescales, revealing ion movements and protein conformational changes relevant to activity. In this chapter we will explore progress in understanding the function of the Na_v family, as well as their drug modulation, using MD simulations of bacterial channels.

SODIUM CHANNEL ARCHITECTURE

Mammalian Na_v channel structure

The mammalian Na_v channel family is composed of 9 known members, Na_v1.1 to Na_v1.9, presenting a high level of homology and sharing a set of common features. In the absence of high-resolution structural data for this family of channels, our understanding of their structure relies on the analysis of their sequence and the interpretation of data obtained through electrophysiology and mutagenesis. Their pore forming subunit is composed of 4 homologous but not identical domains linked together to form a single polypeptide chain. Each domain (DI to DIV) presents a high level of sequence identity throughout the family and is made up of 6 transmembrane helices (S1 to S6), where segments S1 to S4 form the voltage-sensing domain, while S5 and S6 form the pore domain connected together via a cytoplasmic helix (S4–S5 linker). Within the pore domain, segments S5 and S6 are linked together by 2 short helices P1 and P2, and a coil-like structure forming the selectivity filter (Fig.1).

The roles of each segment and their relations to function have been extensively investigated, highlighting the particular importance of segment S4 in voltage sensing [3, 58] and segment S6 in gating and drug binding [59–61]. Similarly, the extra- and intra-cellular flexible loops linking domains and segments have also been the subject of investigation and play specific key roles in the binding of therapeutic agents [62] or for self-regulation [63]. A pattern of conserved residues have been identified in each domain as responsible for the selectivity for sodium, in particular the DEKA locus [64–67], and systematic mutagenesis has shown that these residues need to be in close proximity to maintain the tetrodotoxin binding property characteristic of many Na_v channels [68, 69], which is consistent with the idea of a narrow selective filter.

While this accumulation of functional data and their relation to different parts of the Na_v channels have started to piece together an adequate idea of the structure of the mammalian channels, striking many similarities with other voltage-gated ion channels, such as potassium channels, solution of the X-ray structure of the bacterial channel Na_vAb in 2011 [14] finally offered a complete visualization of channel architecture at the molecular level.

Bacterial Na_v channel structure

Bacterial Na_v channels present a much simpler arrangement than their mammalian counterparts; assembling into homo-tetramers where each subunit is made up of 6 segments. The make up of each subunit closely resembles what is seen in mammalian Na_v channels, with segments S1 to S4 forming the voltage-sensing domain and segments S5 and S6 making up the pore domain (Fig.1). Since the publication of the first X-ray structure of Na_vAb in 2011 [14], subsequent structures, including for Na_vMs [15, 18], Na_vRh [17], Na_vAe [19] and other structures for Na_vAb [16], have confirmed this overall architecture. Taken together, these bacterial channels offer compelling insight into the structure and function relationships for the BacNa_v family.

Overview of the pore domain—Published BacNa_v X-ray structures have been proposed to represent the pore domain in its closed [14, 19], open [15, 18] and inactivated [16, 17] conformations. Despite the proposed disparity of conformations, all structures display remarkably similar pore domains. The BacNa_v pore domain is reminiscent of that of K channels, with a comparable type of folding showing segments S5 and S6 arranged symmetrically around a central axis to form the ion-conducting pore (Fig.2). The transmembrane segments are linked together by 2 pore helices, P1 and P2, and a coil forming the selectivity filter, on the extracellular side of the pore, constituting the P-loop. The existence of a second pore helix, P2, unexpected as it was not seen in K channels, raises questions as to its purpose; though it has been suggested to play a role in coordinating channel activity between the extracellular and intracellular regions of the pore domain [31]. On the cytoplasmic side, helices S6 entangle to form the activation gate, beginning with a wider cavity below the selectivity filter (near the P-loop region), and narrowing towards the intracellular side to form a hydrophobic constriction (Fig.2). This bundle crossing thus creates a long hydrophobic pore (Fig.2), which is largely conserved in diverse ion channels, creating a preferentially stable dewetted pore that can be regulated by external stimuli, such as voltage sensor activation (e.g. [70]), or agonist binding in ligand-gated channels (e.g. [71]).

A most interesting feature of the BacNa_v pore domain is the presence of lateral openings (fenestrations) between the pore and the lipid bilayer [14]. These are formed by adjacent segments S6 at the level of the cavity (Fig.2), and have been shown to allow the passage of lipid tails in both X-ray structures [14, 16] and computational studies [18, 31]. They are also expected to permit the passage of small lipophilic drugs, such as local anesthetic, into the pore and onto a major binding site, blocking the channel; consistent with the proposed mechanisms by Hille in the 1970's [46]. Early studies showed that small hydrophobic molecules can enter and leave the pore even when the cytoplasmic gate is closed [46, 72, 73], suggesting the existence of a pathway into the pore via the membrane. The recent discovery that BacNa_v channels share a similar pharmacology with Na_v channels [18, 21], opens the way to promising perspectives in drug design and understanding of channel modulation by drugs, using atomistic simulations to unveil the binding modes and mechanisms of channel modulation, as we discuss below.

Ion Selectivity filter—While the structure of the pore domain is apparently well conserved between mammalian and bacterial channels, the selectivity filter of BacNa_v is the most striking departure from its mammalian counterpart. Instead of the expected DEKA locus, essential for the selectivity of eukaryotic channels, prokaryotic Na_v channels (with the exception of Na_vRh [17]) display a ring of glutamates EEEE, more commonly associated with Ca_v channels in eukaryotic channels [74] (Fig.3). Despite this discrepancy, BacNa_vs exhibit selective permeabilities similar to eukaryotic Na_vs [9, 32, 75].

The selectivity filter of BacNa_v channels is very different to the well-studied K channels [76]. Where the K channels present a narrow, relatively rigid backbone carbonyl-lined filter, allowing only for a single file of ions and water molecules, the selectivity filter of BacNa_v appears wider and more flexible, suggesting a different selectivity mechanism. Its most distinctive characteristic is the presence of glutamate side-chains inside the pore, lining the filter with mobile and (presumed) negatively charged carboxylates (Fig.2). It is also wide enough to allow the permeation of partially hydrated Na⁺ ions [26, 77]. This specific structure creates at least 3 binding sites for the ions: a 'high field strength' site, S_{HFS}, formed by the side chains of E177, a central site, S_{CEN}, at the level of L176 carbonyls, and an inner site, S_{IN}, created by the carbonyls and hydroxyls of T175 (Fig.2).

It is currently not known if Na⁺ selectivity occurs by a different mechanism in BacNa_v and eukaryotic channels, owing to the distinct EEEE and DEKA signatures, although this has been suggested [32]. This is because the selectivity filter sequence contains other residues that contribute to selective permeation. Mammalian channels also display an additional section of negatively charged D/E residues surrounding the outer mouth of the filter (Fig.3). These additional charged residues help discern Na⁺ [75, 78], such that understanding mammalian Na⁺ selectivity, and how it relates to bacterial selectivity, requires consideration of the entire selectivity filter sequence, not just the DEKA signature, as previously attempted [29]. It remains plausible that these additional residues could cause an asymmetric arrangement of charged side chains that results in a similar high field strength Na⁺-selective site to that observed in BacNa_v.

Voltage sensing domains—The voltage sensing domains are key to the gating process in voltage-gated ion channels, transducing transmembrane electric fields into protein conformational changes that trigger the opening or closing of the ion-conducting pore; essential for the generation and propagation of cellular signals [58, 79]. Voltage-sensing domains share a common architecture throughout all families of voltage-gated ion channels (K_v, Ca_v and Na_v channels), with 4 transmembrane segments, S1 to S4, linked by extra- and intra-cellular short helices and loops of various sizes. These domains carry out signal transduction through movements of positive charges in the S4 helices [80, 81]. Segment S4 carries 4 to 8 positively charged residues, generally arginine but sometimes lysine, separated by 2 hydrophobic residues in a repeated RxxR motif. The domains are connected to the pore domain via a helix-shaped linker located at the cytoplasmic interface between S4 and S5, which transmits the up and down movements of S4 during activation to the gate to cause a lateral opening motion (Fig.2).

Analysis of the sequences and available structures [14, 16, 17] of BacNa_v channels shows similarities in primary structure and folding with K_v voltage sensing domains [79], but in a slightly shorter and simpler version, missing long extra- and intra-cellular loops, and presenting shorter segments. The BacNa_v S4 segment carries 4 highly conserved charged residues R1 to R4 (R99, R102, R105 and R108 in Na_vAb). These positively charged residues can interact with negatively charged clusters of residues carried by segments S1–S3. Two clusters can be identified, the extracellular negatively-charged cluster (ENC, N25, E32 and N49 in Na_vAb) and the intracellular negatively-charged cluster (INC, E59 and D80 in Na_vAb), sitting on both side of the hydrophobic constriction site (HCS, F56 in Na_vAb), which blocks the passage of ions and protons, and confers it its hourglass shape (Fig.2). In the activated conformation, charged residues R1 to R4 interact with the ENC, while in the resting state they are bound to the INC.

In their respective X-ray structures, Na_vAb appears to be almost completely activated [14, 16] while Na_vRh is in a completely activated (or ‘up’) state [17]. Despite the states of their voltage sensing domains, the cytoplasmic gates are closed or collapsed in both channels. This creates a mismatch of linked voltage sensing and pore domains that may impact on the details of those structures. At the bottom of the voltage-sensing domains, a hinge formed by cation- π stacking of a charged residue on the linker (R117 in Na_vAb) and a highly conserved aromatic residue of S3 (W76) seems to play a crucial role in the stabilization of the open/closed states of the gate. This gating hinge contributes to the closed conformation of Na_vAb in particular. The absence of a channel structure with a ‘down’ or resting state configuration of the voltage sensing domain makes it difficult to examine gating motions, though we may learn from other channels [82–84], as we discuss further below.

PRINCIPLES OF SODIUM CONDUCTION AND SELECTIVITY

BacNa_v channels, with their wide selectivity filters, are expected to conduct ions via a different process to the single-file knock-on mechanism now commonly accepted in K channels [85, 86]. Added to this, the signature glutamate side chains that line the upper part of the filter are highly flexible, demonstrated by recent simulations to play active roles in facilitating ion translocation, enabled by this flexibility [27, 31].

The BacNa_v X-ray structures reveal 3 ion binding sites S_{HFS}, S_{CEN} and S_{IN} [14, 17], and an additional site located in the vestibule of the channel S_{OUT} [19], allowing up to 4 ions in the upper part of the pore. Simulations of Na_vAb have shown that the filter typically binds 1 to 4 ions, but with a net preference for the 2- and 3-ion occupancy states [27, 31]. Free energy surfaces describing these two preferred occupancy states reveal a conduction mechanism involving transitions between 2- and 3-ion occupancy (Fig.4), reminiscent of knock-on conduction in K channels [70, 85–87].

In the presence of 2 ions in the selectivity filter, the ions move freely between binding sites S_{HFS}, S_{IN} and S_{CEN}, but seem trapped in the filter in the absence of a 3rd ion (not shown). The entrance of that 3rd ion in the filter reveals an interesting competition of conduction mechanisms, as shown in Fig.4. One possibility involves the upper ion entering the filter from above, joining the lower ion at the ring of glutamates (EEEE), and then knocking the

bottom ion downward, while the alternative is for one ion to pass the other, co-existing at the EEEE ring before pushing the bottom ion into the cavity. These direct knock-on and “pass-by” mechanisms encounter similar low (<1 kcal/mol) free energy barriers, and would both contribute to conduction. Interestingly, the binding of a third ion always involves the rupture of the H-bonds seen between E177 and S178 in the crystal structure [14], leading to a rearrangement of the side-chains of E177, provided sufficient sampling time in simulations.

Ion permeation in Na_vAb can therefore be described as a 3-ion process (Fig.4). During the entire conduction process, the largest free energy barrier encountered is ~ 1.2 kcal/mol ($\sim 2k_B T$), allowing for rapid conduction. This 3-ion mechanism is supported by other recent computational investigations [27] and by previous studies of simplified Glu-lined pores [88], but not in studies that especially focused on a 2-ion process [25, 26]. Other studies have focused on generating ion conduction through a constrained-open pore [15, 28], using large membrane potentials, in contrast to the aforementioned studies that focused on equilibrium sampling, appropriate to physiological voltages, despite using a closed Na_vAb structure.

The specific makeup of the filter and its flexibility are at the center of this dynamic conduction mechanism. The interplay between rotameric changes of the side chains of residues E177 and the movements of permeating Na^+ ions creates an ensemble of distinct bound states, and the rapid exchange between those states is an essential part of the conduction process, allowing for near-diffusion permeation rates in BacNa_v channels (i.e. The flexibility of the side chains, and their ability to undergo structural isomerizations, leads to a continuum of low energy configurations connecting distinct free energy minima, facilitating conduction by lowering activation barriers [27, 31]). Such intimate involvement in ion conduction is only achieved when simulating on the timescales of conduction itself, and is thus only adequately sampled through 100^3 of nanoseconds or more of simulation, not achieved in early studies [25, 26], but adequately in subsequent simulations [27, 31].

In contrast to the narrow, ion-sized selectivity filter of the K channel, the BacNa_v filter is wider and lined not only by protein backbone (residues 175 to 178), but also by the side chains of a ring of 4 glutamates (E177) (Fig.2). Curiously, this ring is conserved within the BacNa_v family, but is indicative of a Ca^{2+} -selective [74] and not of a Na^+ -selective mammalian channel, which has a conserved DEKA locus [35] (Fig.3). Despite the EEEE-lined selectivity filter, bacterial channels nominally favor Na^+ over Ca^{2+} or K^+ , with relative permeabilities $5 < P_{\text{Na}}/P_{\text{K}} < 171$ and $7 < P_{\text{Na}}/P_{\text{Ca}} < 70$ in NaChBac [9, 32, 75]. This Na^+ -selectivity emerging from a Glu-lined pore leads us to question the mechanism by which these channels optimally conduct Na^+ ions.

Simple physical models have proposed a charge/space competition theory to explain the variable $\text{Na}^+/\text{Ca}^{2+}$ selectivity of Glu-lined selectivity filters, where the volume and electrostatic environment both play a role in the number of ions that the channels can bind and efficiently conduct [75, 88, 89]. It has been suggested [90] that confinement of charged ligands in a smaller volume will favor the binding of divalent ions over monovalent ions. Another factor is the extent of additional negative charges in the vicinity of the selectivity filter, with increased number expectantly increasing propensity for attracting Ca^{2+} [75]. Clearly molecular simulations are needed to better explore valence selectivity in the sodium

channel. A major hindrance has been the availability of accurate models of Ca^{2+} ions to capture the interactions with water, protein carboxylates and carbonyl groups [91], with recent attempts observing significant over-binding of the divalent ions [31].

To understand competition between the monovalent cations Na^+ and K^+ in the sodium channel, we can learn much by returning to the case of K^+ selectivity in a K channel. A long-running hypothesis is that the K channel thermodynamically chooses to bind K^+ over Na^+ ions with greater affinity [92] (although this apparent fact has been recently challenged, and discussed further below [33]). While it has been argued that size alone might achieve such difference in thermodynamic stability [93], this has been disputed simply because proteins are highly flexible, with even their backbones fluctuating on the Å-scale. These fluctuations determine the flexible response of the coordinating ligands that control ion binding [94, 95], deeming sub-Å differences in ion size of little energetic consequence. While there have been suggestions of such discrimination based on small differences in the coordinating environments of Na^+ and K^+ within the Na_v filter [25, 26], flexible response is an even more important contributor in the sodium channel, due to its dynamic coordinating glutamate side chains, leading not only to strong Na^+ binding, but a highly efficient Na^+ conduction mechanism.

We may also learn by comparing the nature of the coordinating ligands between Na and K channels. Selectivity between the monovalent cations Na^+ and K^+ may be interpreted in terms of ligand field strength [96], where amide carbonyls provide weaker fields that favor larger ions, like K^+ , whereas carboxylates create a high field strength site favorable to Na^+ . Yet in both the sodium and potassium channels, selectivity is an inherently dynamic process [34], rather than governed strictly by the geometry and chemical nature of the ligands encountered by the ions. Furthermore, despite the likely effects of ligand chemistry and dynamics on the binding of ions, we must acknowledge that selective permeation of the channels is ultimately a kinetic process [97]. In a K channel, selectivity has been shown to arise from a well-optimized multiple-ion conduction mechanism, where K^+ ions encounter low free energy barriers to permeation. This mechanism breaks down in the presence of Na^+ , as those ions bind to different (though adjacent) sites, disrupting the low free energy pathways [34]. This kinetic view of selective exclusion of ions suggests that one can only truly observe and understand selectivity by examining the free energy landscape governing the movements of all of the ions in the pore.

For the sodium channel, we have also observed that the multi-ion scheme is a highly optimized knock-on mechanism, for Na^+ ions [27, 31]. In this process we have observed how essential E177 rotamer changes and the formation of ionic clusters with their carboxylate groups are to efficient permeation. We must then turn to examine the behavior of K^+ ions in the sodium channel to begin to learn about its selectivity process. Preliminary results obtained during MD simulations of the permeation of K^+ ions in Na_vAb show that this ion is unable to form the same kind of interactions with E177 [31], and that reduced clustering of K^+ with those side chains leads to an overall reduction in ion occupancy [31]. Yet, simulations in the presence of K^+ ions indicate that the free energy surface for a reduced 1–2 ion knock-on mechanism may still permit conduction of K^+ ions, albeit at a reduced rate (Boiteux *et al.*, In preparation). Key to the difference thus appears to be the ability of Na^+ to

form a 3-ion occupancy state of the filter due to stronger interactions with charged carboxylates.

What we have learned so far from simulations of bacterial channels can aid us in describing the likely situation in the mammalian channel, with its distinct DEKA locus in the selectivity filter. The DEKA signature does indeed contain 2 carboxylates that may bind sodium ions in a similar fashion to the bacterial channel. We again note that the selectivity filter contains outer rings of charged residues (Fig.3) that may contribute to the binding of multiple ions (as suggested by their mutagenesis [75]). What then is the role of the lysine residue? It is known experimentally that its mutation affects selectivity in mammalian channels [65, 98]. It has been suggested theoretically that this Lys may act to repel Na^+ ions towards the opposing D and E residues [29]. However, it is also possible that simulations on the timescales of permeation may reveal that the asymmetric channel and flexible D and E (and potentially outer ring carboxylates) provide plenty of binding partners for a multi-ion mechanism involving Na^+ , as well as for the charged (or neutralized) Lys within the pore. Computational work on mammalian models, based on BacNa_v templates, should be helpful in explaining Na^+ selectivity in mammalian channels.

STRUCTURAL BASIS FOR ACTIVATION AND INACTIVATION

Activation/gating

There has been much progress in understanding voltage sensing movements in the family of voltage-gated ion channels in general, allowing us a reasonable understanding about this process for the sodium channels [36], with changes in the voltage sensing and pore domains described above. At this point in time, published X-ray structures have been proposed to represent the pore domain in its closed [14, 19], open [15, 18] and inactivated [16, 17] conformations; seemingly offering structural definitions of the important states in the function of the channel.

Gating motions in the pore domain of bacterial Na_v channels have been a matter of some contention. The initial unavailability of an open state BacNa_v meant that studies of ion conduction were forced to simulate a closed pore based on Na_vAb . The proposal of an open state form of BacNa_v [15, 18] did not uncover the degree of conformational change and pore opening anticipated. Comparison of pore radii in Fig.5 shows very little difference at the level of the cytoplasmic gate, suggesting a strong bias toward a closed pore, even if the S5 and S6 arrange themselves slightly differently in these structures. In fact, Fig.5 suggests the first published Na_vMs (proposed open) structure [15] is just as closed at the previous structures. In the most recent Na_vMs structure, we now see a small deflection at the very bottom in Fig.5, corresponding only to deflection of the end of S6 in PDB:4CBC [18].

The marginal change observed when comparing closed and proposed open channels, has led to the proposition of a subtle gating mechanism involving a slight bend at Thr209 on S6 [15]. Whether this bend is sufficient to constitute a distinct conformation on the free energy surface has yet to be determined. Moreover, simulations have indicated this may merely correspond to a natural fluctuation of the pore within the one state [31] (although also appears linked to changes proposed to be linked to inactivation – see below). Nevertheless,

unless heavily constrained to stay open [28], simulations of this pore domain-only structure, either isolated or used as a template to build more complete open models of Na_vAb or Na_vRh involving the voltage sensing domains, have been unsuccessful in maintaining an open pore (Boiteux and Allen. Unpublished). A significant part of the problem lies with the absence of voltage-sensing domains in the Na_vMs structure, and the issues in merging with the complete structures of Na_vAb or Na_vRh, which have been solved in a ‘pre-active’ states with fully or partly activated voltage-sensing domains and closed pores. Such a mismatch means the interactions of the protein and its S4-S5 linker with S6 may be unfavorable. Solution of a complete channel (pore and voltage sensor domains) in an open state remains an important challenge in the field.

Inactivation

Na_v channels cycle through resting, open and inactivated states during their function. They typically open on depolarization and close either rapidly upon repolarization, or more slowly on sustained depolarization. The latter mechanism is called inactivation and causes a refractory period during which channels remain unresponsive to further depolarization. This refractory period, during which neurons would become unresponsive to depolarization, was observed as early as 1924 [99], and a long, sustained pulse had to be applied to restore excitability. This phenomenon was later on associated with specific characteristic of Na⁺ channels, when Hodgkin and Huxley first described neuron excitability in terms of sodium and potassium conductance, and identified an inactive state for the channels [1, 100].

Inactivation occurs on different timescales [101, 102], leading to at least two different classes: fast and slow inactivation, also called N-type and C-type; terms initially coined to describe inactivation in the Shaker K channel [103]. Inactivation is an essential physiological process, protecting cells from over-excitability, which has been associated with periodic paralysis [104, 105], arrhythmia [106], long QT syndrome [107] or epilepsy [108, 109] among others issues.

Fast inactivation—Fast inactivation in eukaryotic Na_v channels typically occurs during sustained depolarization on a timescale of the order of a millisecond (varying among channel isoforms [110]) via a “ball and chain” or “hinged lid” mechanism during which a cytoplasmic part of the protein (the ball) obstructs the pore by binding to a docking site. The inactivating particle has been identified as the cytoplasmic loop linking DIII and DIV [63, 111], in particular 3 highly conserved residues forming the IFM motif [112–114]. The docking site is made up of numerous cytoplasmic regions surrounding the gate, including the linker S4-S5 of domains III and IV [115] and the cytoplasmic terminus segment S6 in DIV [116]. Fast inactivation plays an important role for action potential repolarization, in particular within the nodes of Ranvier [117].

In the absence of cytoplasmic linker between subunits in prokaryotic Na_v channels [14], they display no fast inactivation and knowledge of their structure offers limited additional insight into this mechanism for mammalian channels. However, MD studies of homology models have been used to examine flexible linker interactions with both the pore and voltage sensors, to provide insight into the fast inactivation process [118]. While both fast and slow

inactivation play roles in function and modulation (e.g. [42, 119]), the simplicity of the bacterial channels allows for experimental and computational studies to easily focus on the one process of pore-based slow inactivation, as done with K channels [45]. This enables molecular-level studies of inactivation with simulations based on bacterial channel structures.

Slow inactivation—Slow inactivation is distinct from fast inactivation and does not involve the cytoplasmic linker specific to eukaryotic channels. It is a less mechanically defined process determined to involve the region of the pore. Slow inactivation has no clearly defined timescale, and can span multiple orders of magnitude, from milliseconds to minutes [120, 121].

Structural investigations have shown that different pore regions can be associated with slow inactivation (Fig.6). In particular, mutations around the selectivity filter (rNa_v1.4 DI-selectivity filter W402C [122, 123] and W402A [124], equivalent W179 in Na_vAb, DI-selectivity filter E403C [125], aligned with S180 in Na_vAb, and DIII-selectivity filter F1236C [126], aligned with L176 in Na_vAb) and the segments S6 forming the cytoplasmic gate (rNa_v1.4 DI-S6 N434A [127], equivalent N211 in Na_vAb, DII-S6 V787C [128], aligned with Na_vAb M209, DIV-S6 V1583C [129], DIV-S6 I1575A [130], equivalent I202 in Na_vAb and hNa_v1.5 DIV-S6 F1760A [53]) can modulate slow inactivation rates. The distribution of the residues potentially involved, or located near conformational changes involved in slow inactivation, signify a complex and poorly understood process. The regions suggest linking of the selectivity filter and segment S6 in a potential collapse of large scale throughout the pore domain.

Prokaryotic Na_v channels display some form of inactivation (necessarily pore-based), occurring on the millisecond to seconds timescale [9, 16, 17] that is compatible with timescales described for slow inactivation in mammalian Na_v channels. Additionally, structures of potentially inactivated or partially inactivated states of Na_vAb [16] and Na_vRh [17] show an asymmetrically collapsed gate, involving bonding between L123 (linker S4-S5) and S6 N211 (equivalent N434 involved in slow inactivation in Na_v1.4 [127]) in Na_vAb [16]. Another common feature of these inactivated structures is a narrower selectivity filter, described as closed in Na_vRh [17]. However this closed selectivity filter was shown to spontaneously open during MD with only a slight re-orientation of the side chains of the residues lining it but remained poorly conductive [30]. The structural data collected in eukaryotic Na_v channels is consistent with the conformational changes observed in BacNa_v channels, suggesting that they share a common mechanism for slow inactivation, further suggesting bacterial channels are good models for understanding the process in mammalian channels.

While equilibrium with a true slow inactivated state is not likely to be achieved on accessible timescales using MD simulation, recurrent behavior relevant to inactivation can be sampled. As described, Na_vAb's selectivity filter is very flexible, and this appears to be key to conduction and selectivity [27, 31]. Interestingly, studies on long timescales have shown that conformational changes within the filter are correlated with significant distortion along segments S6 [31], coincident with pore collapses that are much akin to the proposed

inactivated forms of the channel [16, 17] (see Fig.7). Analysis of the backbone fluctuations in the segments S6 of Na_vAb shows some instability between residues V196 and P200, in the top part of the helices, and around residues I202 and F203, located in the middle of S6, at the level of the fenestrations. The structure of S6 depends strongly on the state of 2 concurrent I202–T206 interactions: the backbone to backbone helix forming H-bonding and the interfering backbone to side chain H-bond, potentially helix-weakening. While serine is more notably described as a helix-breaker [131, 132], threonine displays a similar mechanism of interfering with α -helical structure and is also known to modulate the bending commonly induced by proline in α -helices [133]. In Na_vAb, the methyl group of residue T206 is surrounded by a large cluster of nonpolar side chains from neighboring residues that helps direct the hydroxyl group toward the carbonyl oxygen of I202, leading to weakening of the α -helix forming H-bond. This helix-weakening effect around I202 and T206 is reinforced by the significant deformation introduced by residue P200 between F198 and I202, leading to deep bending of segment S6. In the most extreme cases, this bending is associated with a tilt of P2 that pulls on the selectivity filter from above and contributes to large-scale selectivity filter distortion.

Taken together, these conformational changes result in protein structures that resemble the proposed inactivated structures of Na_vAb and Na_vRh [16, 17] (Fig.7). These structures present asymmetrically collapsed S6 activation gates (asymmetry of 5 Å at the level of residues 215–218 for Na_vAb, but only ~1 Å for Na_vRh), as well as an associated narrower selectivity filter and a reshaped cavity. During simulations, similar gate asymmetry has been observed, reaching as high as 12 Å, and a narrower to collapsed selectivity filter [31].

This bending of segment S6 appears to be a natural feature of the channel. Significantly, it can be linked to a cluster of key residues located at the level of the cavity fenestrations and associated with slow inactivation in both mammalian and bacterial channels (see Fig.8). Mutation of the helix-weakening T206, described as the hinge point in open Na_vMs [15], has been shown to prevent inactivation in NaChBac (T220) [134], while mutation of the highly conserved equivalent to I202 dramatically alters slow inactivation in Na_v1.4 [130]. Moreover, mutation of a phenylalanine at that same level (not equivalent but in similar position and orientation to F203 in Na_vAb) has been associated with a strong inhibition of slow inactivation in mammalian channels [53]. While these rapid conformational changes would need to be stabilized on much longer timescales (ms to s) to yield a truly slow inactivated channel, they give some significant insights into the structural changes occurring during the transition from a conducting to an inactivated state. It is also of much interest that changes in S6 helices are correlated with conformational changes in the filter, a phenomenon described during C-type (slow) inactivation in K channels [45], and suggested by NaChBac studies.

DRUG BINDING AND MODULATION

Na_v channels are involved in a vast range of pathologies, including neurological, muscular and cardiac disorders [4, 7, 8, 135–138]. As such, they're the primary targets for many drugs, such as local and general anesthetics, anti-epileptics or anti-arrhythmics among others [7, 138, 139]. The modulation of Na_v channels by pharmacological agents is a complex

phenomenon, involving voltage- and state-dependent block of current, specific interaction with various binding sites in different activated and inactivated states, and modification of the recovery time from these inactivated states [3]. It has long been postulated that the fast-inactivated state was the conformational target for state specific binding [46, 140, 141], and local anesthetics, for example, can see their potency reduced in fast-inactivation defective channels [142–144] while anti-epileptic drugs are believed to restore fast-inactivation in deficient channels [37]. However, the focus has recently begun to include slow inactivation, as both local anesthetics and anti-epileptics have been shown to modulate slow-inactivated channels [44, 145–148], and mutation of key residues forming the receptor site for local anesthetics affects slow inactivation [53].

Functional studies have successfully identified 2 major binding sites for local anesthetic and anti-epileptic agents: a high affinity site, that appears to be state and voltage dependent; and a low affinity site apparently involving closed channels [149, 150]. The high affinity site has been the subject of extensive investigation [139, 151–153] since its first identification in 1994 [154] and appears to be common to many drugs [59, 152]. This site has been located on segment S6 of domain DIV, and involves a phenylalanine located in the middle of the helix (rNa_v1.2 F1764 [154] and hNa_v1.5 F1760 [53, 151]) and a tyrosine belonging to the lower half of the helix (rNa_v1.2 Y1771 [154] and hNa_v1.5 Y1766 [151]). The low-affinity site is not as well characterized and has been described as resulting from a “combination of hydrophobic interactions” occurring when the channel is maintained at hyperpolarized voltages [150].

Despite this recent accumulation of knowledge about the binding of drugs, there is still little known about the mechanism by which these drugs alter the functions of sodium channels. The development of new therapeutic agents for specific disorders, while limiting or avoiding altogether possible side effects, will rely on our ability to understand the complex interactions controlling selective drug binding to Na_v channels. Bacterial Na_v channels can be considered models for understanding drug binding as they display sensitivity to common Na_v inhibitors such as [18, 21, 49, 155], and key binding regions are well conserved between mammalian and bacterial channels [14, 18]. *In silico* studies of BacNa_v channels in the presence of local anesthetics and anti-epileptics have been successfully used to shed light on binding sites and inhibition mechanisms [18, 47, 48]. Additionally, crystal complexes of brominated drug-like compounds bound to the Na_vMs channel have shown binding in the cavity near what appears to be the high affinity site [18].

Access and binding to the central cavity and high-affinity site

MD simulations of the local anesthetic benzocaine [47] (as well as other local anesthetic and anti-epileptic drugs; Boiteux *et al.* In preparation) show extensive binding in the hydrophobic cavity, and more specifically to residue F203, consistent with its role as the high-affinity binding site in Na_vAb. The simulations reveal two different access pathways, via the lipid membrane through the fenestrations, and, unexpectedly, directly through the cytoplasmic gate despite it being closed (benzocaine) (Fig.9). The coexistence of these 2 access routes is in excellent agreement with the hydrophobic (fenestration) and hydrophilic

(gate) pathways predicted by Hille [46], but never before demonstrated at the molecular level.

Na_vAb may offer unique similarity to mammalian Na_v channels for the investigation of drug binding within the high affinity pore site, because of the presence of the pore-facing F203. Long unbiased MD simulations [47] and Metadynamics simulations [48] of benzocaine and anti-epileptic phenytoin have suggested binding in the cavity of Na_vAb, having preferential interactions with F203, following passage through fenestrations to this high affinity site. Other bacterial Na_v channels only possess Phe residues on S6 that are directed away from the pore [47]. Interestingly, however, binding may still occur in the absence of this residue, as demonstrated for brominated drug-like compounds to more distant F214 on S6 in Na_vMs [18]. In this case, docking of known Na_v antagonists showed preferential binding in the top half of the cavity and around the selectivity filter, rather than through the fenestrations [18]. A study of a model of NaChBac in the presence of the general anesthetic isoflurane has also shown access to the cavity via the fenestrations, though suggests binding to a phenylalanine that sits low on the S6 helix (F227, equivalent Y1771) [155], despite the suggestion that no Phe side chain on NaChBac is capable of facing the pore [47]. The shared binding in the pores of different bacterial (and mammalian) channels suggests a dominant binding in a hydrophobic pore region, influenced by available aromatic and polar groups in each protein.

Binding around the cytoplasmic gate and the linker

The anti-epileptic drug phenytoin [47] binds to residue N211 at the level of the cytoplasmic gate, between segments S6 and the linkers S4–S5 (Fig.9). In the case of phenytoin, the drug interacts via H-bonding with N211 and D219 of adjacent subunits, effectively keeping the gate in its closed state. For both drugs, this binding site forms a wedge between the outside of the gate and the linker, and would be more accessible when the voltage-sensing domain is in an activated state, lifting the linker up, and when the gate is closed or collapsed; a conformation compatible with a slow-inactivated state. Binding at this site would be expected to stabilize this conformation. Similar results were obtained during the simulation of isoflurane with NaChBac, showing binding in the same location with N425 (equivalent to N211 in Na_vAb) [155]. Mutation of the equivalent of residue N211 in channel rNa_v1.4 (DI-S6 N434A [127]) has been shown to modulate slow inactivation, reinforcing the significance of binding at this site for anti-epileptic drugs that target and enhance slow inactivation [44, 51, 148, 156].

Interactions within voltage sensing domains

While direct interaction with the pore domain seems the most intuitive inhibition process, other modulating mechanisms should not be disregarded. Channel inhibitors are known to alter the voltage sensor to gate coupling through various processes, either by preventing the recovery from an inactivated state, or by stabilizing or actively inducing a non-conductive state [139]. Local anesthetics in particular have been shown to affect the voltage sensors in domains III and IV of Na_v channels, disrupting their coupling with the pore domain and rendering the channel insensitive to voltage [157]. During simulations, all investigated drugs display some level of interaction with the voltage-sensing domains, and in particular with the charged residues of S4. Benzocaine is seen interacting via cation- π stacking with R2,

phenytoin forms H-bonds with R4 (Fig.9), while other drugs have been seen to interact with both R2 and R4, as well as R3 (not shown; Boiteux et al. In preparation).

Drugs such as local anesthetics and anti-epileptics are known to modulate the response of Na_v channels to voltage [157], but this effect is usually considered as an indirect consequence of the binding of the drugs around S6 and subsequent effect on the gate and permeation of ions. While some toxin binding sites have been clearly identified at the top of some of the voltage-sensing domains [62], the direct interaction of drugs with specific residues of Na_v is not well documented. However, a study has recently shown that the anti-epilepsy drug ztz240 interacts directly with the charged residues of S4 and the conserved Phe of the HCS (S2) of $\text{K}_v7.2$ to modulate its activation [158]. No such results can be found for Na_v channels but the high level of conservation of both the charged residues of S4 and the Phe forming the HCS suggests that the same kind of interaction can be expected in Na_v channel.

The effects of such interactions remain difficult to interpret and would be highly state dependent. In the activated conformation, residues R2 and R3 would be more exposed and likely to become attractive binding sites, the up conformation would then be stabilized and the channel rendered less sensitive to hyperpolarization and subsequent depolarizations. Such a phenomenon is consistent with the effects of a drug targeting and enhancing slow inactivation. We shall be better positioned to use MD simulation to understand voltage sensor contributions to drug modulation, once an X-ray structure of a down state voltage sensor has been solved.

DISCUSSION AND CONCLUSIONS

While experimental techniques can probe the structures and activities of ion channels, they remain limited by experimental length- and time-scales, and their abilities to observe the detailed motions and interactions of the atoms that give rise to channel function. In the past few years, breakthroughs in several atomic structures of Na_v channels have provided the necessary springboard from which we can begin to explore function using atomistic simulations. In this chapter we have described recent developments in the use of structurally-defined bacterial channels, in conjunction with modern atomistic MD simulations that push towards physiological timescales. These studies have, for the first time, uncovered molecular-level mechanisms underlying neuronal signaling, and how they may be modulated by different molecules.

The solution of atomic-resolution structures for Na^+ -selective ion channels has enabled computer simulations to shed light on Na^+ conduction mechanisms, and the ability of the protein to discriminate against other physiologically abundant cations. Although the bacterial channels exhibit a ring of glutamates that is distinct from the mammalian signature sequence (involving glutamate, aspartate, lysine and alanine), those simulations revealed how carboxylate groups preferentially attract Na^+ and facilitate low-barrier ion translocation across a membrane channel. Simulations demonstrated a different mechanism to that we had become familiar with in K channels, where a regular single-file movement of multiple K^+ ions passes through a conformationally rigid selectivity filter. In the sodium channel, the

filter itself is an active participant, with flexible Glu side chains forming complexes with multiple ions, and essentially carrying the ions across the narrow constriction. This conduction is either via a familiar knock-on mechanism, or via a pass-by mechanism where the high affinity for the Glu site enables concurrent binding of two Na^+ ions. Simulations have suggested that this does not occur for the larger K^+ , with lower affinity for the high field strength site, and reduced overall occupancy. The challenge now remains to create more accurate empirical models of the also abundant Ca^{2+} ion, to resolve the mystery of why a Glu lined filter is Na^+ -selective in one organism, and Ca^{2+} selective in another. The further challenge remains to extrapolate these findings to understand mammalian selectivity mechanisms, through carefully constructed homology models or through breakthroughs in eukaryotic channel crystal structures.

Atomic simulations have also shed light on the possible conformational changes in the sodium channel, and how they may relate to activation and inactivation mechanisms. Simulations have reported significant protein conformational flexibility on the multi- μs timescale, influencing the conducting state of the pore, as well as its interplay with the lipid membrane. Simulations have observed pore collapse, involving bending of helix S6, leading to an asymmetric arrangement that resembles the inactivated state proposed experimentally. These changes have been shown to involve residues that are critical to slow inactivation in bacterial and mammalian channels. They also reveal communication throughout the protein, through S6 and the P2 helix of the P-loop, and steric interactions with the selectivity filter itself. They also are associated with the opening and closing of membrane-bound fenestrations, giving clues as to the processes that underscore inactivation and drug inhibition of mammalian Na_v channels.

It is known experimentally that drugs influence both slow and fast inactivation in mammalian sodium channels, and that these drugs inhibit bacterial channels in a similar fashion; targeting pore-based slow inactivation. This motivates the use of newly solved crystal structures to explore the binding of local anesthetic, anti-epileptic and general anesthetic molecules to bacterial channels, as simplified models of human sodium channels. Simulations of neutral, hydrophobic drugs, revealed high affinity pore-blocking, approached via paths that were either lipid-bound fenestrations, or through a closed intracellular gate. In Na_vAb , the high affinity site involves a Phe side chain that mimics the side chain that controls binding in mammalian channels, while in other bacterial channels, binding is still achieved inside the pore of the channel with similar affinity. A range of lower affinity sites have been identified, which are expected to modulate channel function by altering the stability of different states of the gate (with different size and asymmetry), communication of change between voltage sensors and the gate, or the voltage sensor movements themselves. Evidence suggests that these binding sites are dependent on the chemical nature of the drug, suggesting that such extensive sampling of binding pathways and sites is required for each drug candidate.

These recent studies represent the first stage in understanding the molecular origins of sodium channel inhibition. More complete investigations must focus on the state dependence of binding, and ultimately capture the modulation of the receptor by the shifting of the equilibrium of conformational states, presenting a significant challenge to atomistic MD

simulation. These studies have, however, demonstrated that simpler bacterial channels offer a wealth of mechanistic information that can be used to learn modulation processes in humans, and provide new targets for therapeutically selective sodium channel drugs.

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REFERENCES

1. Hodgkin AL and Huxley AF, A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol*, 1952 117(4): p. 500–44. [PubMed: 12991237]
2. Goldin AL, Diversity of mammalian voltage-gated sodium channels. *Ann N Y Acad Sci*, 1999 868: p. 38–50. [PubMed: 10414280]
3. Hille B, Ionic channels of excitable membranes. 3rd ed 2001: Sunderland, Sinauer.
4. Ashcroft F, Ion Channels and Disease. 2000, University of Oxford, UK: Elsevier.
5. Clare JJ, Targeting voltage-gated sodium channels for pain therapy. *Expert Opin Investig Drugs*, 2010 19(1): p. 45–62.
6. Overington JP, Al-Lazikani B, and Hopkins AL, How many drug targets are there? *Nat Rev Drug Discov*, 2006 5(12): p. 993–6. [PubMed: 17139284]
7. Kyle DJ and Ilyin VI, Sodium channel blockers. *J Med Chem*, 2007 50(11): p. 2583–8. [PubMed: 17489575]
8. Zuliani V, et al., Recent advances in the medicinal chemistry of sodium channel blockers and their therapeutic potential. *Curr Top Med Chem*, 2009 9(4): p. 396–415. [PubMed: 19442209]
9. Ren D, et al., A prokaryotic voltage-gated sodium channel. *Science*, 2001 294(5550): p. 2372–5. [PubMed: 11743207]
10. Koishi R, et al., A superfamily of voltage-gated sodium channels in bacteria. *J Biol Chem*, 2004 279(10): p. 9532–8. [PubMed: 14665618]
11. Ito M, et al., The voltage-gated Na⁺ channel Na_vBP has a role in motility, chemotaxis, and pH homeostasis of an alkaliphilic *Bacillus*. *Proc Natl Acad Sci U S A*, 2004 101(29): p. 10566–71. [PubMed: 15243157]
12. Payandeh J and Minor DL Jr., Bacterial Voltage-Gated Sodium Channels (BacNas) from the Soil, Sea, and Salt Lakes Enlighten Molecular Mechanisms of Electrical Signaling and Pharmacology in the Brain and Heart. *J Mol Biol*, 2015 427(1): p. 3–30. [PubMed: 25158094]
13. Sato C, et al., The voltage-sensitive sodium channel is a bell-shaped molecule with several cavities. *Nature*, 2001 409(6823): p. 1047–51. [PubMed: 11234014]
14. Payandeh J, et al., The crystal structure of a voltage-gated sodium channel. *Nature*, 2011 475(7356): p. 353–8. [PubMed: 21743477]
15. McCusker EC, et al., Structure of a bacterial voltage-gated sodium channel pore reveals mechanisms of opening and closing. *Nature Comm*, 2012 3: p. 1102.
16. Payandeh J, et al., Crystal structure of a voltage-gated sodium channel in two potentially inactivated states. *Nature*, 2012 486(7401): p. 135–9. [PubMed: 22678296]
17. Zhang X, et al., Crystal structure of an orthologue of the NaChBac voltage-gated sodium channel. *Nature*, 2012 486(7401): p. 130–134. [PubMed: 22678295]
18. Bagneris C, et al., Prokaryotic Na_vMs channel as a structural and functional model for eukaryotic sodium channel antagonism. *Proc Natl Acad Sci USA*, 2014.
19. Shaya D, et al., Structure of a prokaryotic sodium channel pore reveals essential gating elements and an outer ion binding site common to eukaryotic channels. *J Mol Biol*, 2014 426(2): p. 467–83. [PubMed: 24120938]
20. Vilin YY and Ruben PC, Slow inactivation in voltage-gated sodium channels: molecular substrates and contributions to channelopathies. *Cell Biochem Biophys*, 2001 35(2): p. 171–90. [PubMed: 11892790]

21. Lee S, Goodchild SJ, and Ahern CA, Local anesthetic inhibition of a bacterial sodium channel. *J Gen Physiol*, 2012 139(6): p. 507–16. [PubMed: 22641643]
22. McArthur JR, et al., Interactions of key charged residues contributing to selective block of neuronal sodium channels by mu-conotoxin KIIIA. *Mol Pharmacol*, 2011 80(4): p. 573–84. [PubMed: 21709136]
23. Finol-Urdaneta RK, et al., Polymodal, High Affinity Actions of mu-Conotoxins on a Bacterial Voltage-Gated Sodium Channel. *Biophys J*, 2013 104(2): p. 136a–137a.
24. Amaral C, et al., Exploring conformational states of the bacterial voltage-gated sodium channel Na_vAb via molecular dynamics simulations. *Proc Natl Acad Sci U S A*, 2012 109(52): p. 21336–41. [PubMed: 23150565]
25. Corry B and Thomas M, Mechanism of ion permeation and selectivity in a voltage gated sodium channel. *J Am Chem Soc*, 2012 134(3): p. 1840–6. [PubMed: 22191670]
26. Furini S and Domene C, On conduction in a bacterial sodium channel. *PLoS Comput Biol*, 2012 8(4): p. e1002476. [PubMed: 22496637]
27. Chakrabarti N, et al., Catalysis of Na⁺ permeation in the bacterial sodium channel Na_vAb. *Proc Natl Acad Sci U S A*, 2013 110(28): p. 11331–6. [PubMed: 23803856]
28. Ulmschneider MB, et al., Molecular dynamics of ion transport through the open conformation of a bacterial voltage-gated sodium channel. *Proceedings of the National Academy of Sciences of the United States of America*, 2013 110(16): p. 6364–6369. [PubMed: 23542377]
29. Xia M, et al., The mechanism of Na⁺/K⁺ selectivity in mammalian voltage-gated sodium channels based on molecular dynamics simulation. *Biophys J*, 2013 104(11): p. 2401–9. [PubMed: 23746512]
30. Zhang X, et al., Analysis of the selectivity filter of the voltage-gated sodium channel Na_vRh. *Cell Res*, 2013 23(3): p. 409–22. [PubMed: 23247626]
31. Boiteux C, Vorobyov I, and Allen TW, Ion conduction and conformational flexibility of a bacterial voltage-gated sodium channel. *Proc Natl Acad Sci USA*, 2014 111(9): p. 3454–9. [PubMed: 24550503]
32. Finol-Urdaneta RK, et al., Sodium channel selectivity and conduction: prokaryotes have devised their own molecular strategy. *J Gen Physiol*, 2014 143(2): p. 157–71. [PubMed: 24420772]
33. Kim I and Allen TW, On the selective ion binding hypothesis for potassium channels. *Proc Natl Acad Sci U S A*, 2011 108(44): p. 17963–8. [PubMed: 22011574]
34. Thompson AN, et al., Mechanism of potassium-channel selectivity revealed by Na⁺ and Li⁺ binding sites within the KcsA pore. *Nat Struct Mol Biol*, 2009 16(12): p. 1317–24. [PubMed: 19946269]
35. Charalambous K and Wallace BA, NaChBac: the long lost sodium channel ancestor. *Biochemistry*, 2011 50(32): p. 6742–52. [PubMed: 21770445]
36. Yarov-Yarovoy V, et al., Structural basis for gating charge movement in the voltage sensor of a sodium channel. *Proc Natl Acad Sci USA*, 2012 109(2): p. E93–102. [PubMed: 22160714]
37. Rogawski MA and Loscher W, The neurobiology of antiepileptic drugs for the treatment of nonepileptic conditions. *Nat Med*, 2004 10(7): p. 685–92. [PubMed: 15229516]
38. Kaczorowski GJ, et al., Ion channels as drug targets: the next GPCRs. *J Gen Physiol*, 2008 131(5): p. 399–405. [PubMed: 18411331]
39. Goldin AL, Mechanisms of sodium channel inactivation. *Curr Opin Neurobiol*, 2003 13(3): p. 284–90. [PubMed: 12850212]
40. Pavlov E, et al., The pore, not cytoplasmic domains, underlies inactivation in a prokaryotic sodium channel. *Biophys J*, 2005 89(1): p. 232–242. [PubMed: 15849254]
41. Capes DL, et al., Gating transitions in the selectivity filter region of a sodium channel are coupled to the domain IV voltage sensor. *Proc Natl Acad Sci U S A*, 2012 109(7): p. 2648–53. [PubMed: 22308389]
42. Fozzard HA, Lee PJ, and Lipkind GM, Mechanism of local anesthetic drug action on voltage-gated sodium channels. *Curr Pharm Des*, 2005 11(21): p. 2671–86. [PubMed: 16101448]

43. Zeng Z and French C. The modulation of sodium conductance by phenytoin is mediated by slower rather than fast inactivation processes, and is unaffected by fast inactivation removal. in Australian Neurosciences Society 2011 Auckland.
44. Errington AC, et al., The investigational anticonvulsant lacosamide selectively enhances slow inactivation of voltage-gated sodium channels. *Mol Pharmacol*, 2008 73(1): p. 157–69. [PubMed: 17940193]
45. Cuello LG, et al., Structural mechanism of C-type inactivation in K^+ channels. *Nature*, 2010 466(7303): p. 203–8. [PubMed: 20613835]
46. Hille B, Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. *J Gen Physiol*, 1977 69(4): p. 497–515. [PubMed: 300786]
47. Boiteux C, et al., Local anesthetic and antiepileptic drug access and binding to a bacterial voltage-gated sodium channel. *Proc Natl Acad Sci U S A*, 2014 111(36): p. 13057–62. [PubMed: 25136136]
48. Martin LJ and Corry B, Locating the route of entry and binding sites of benzocaine and phenytoin in a bacterial voltage gated sodium channel. *PLoS Comput Biol*, 2014 10(7): p. e1003688. [PubMed: 24992293]
49. Barber AF, et al., Modulation of a voltage-gated Na^+ channel by sevoflurane involves multiple sites and distinct mechanisms. *Proc Natl Acad Sci USA*, 2014 111(18): p. 6726–31. [PubMed: 24753583]
50. Li HL, et al., A molecular basis for the different local anesthetic affinities of resting versus open and inactivated states of the sodium channel. *Mol Pharmacol*, 1999 55(1): p. 134–41. [PubMed: 9882707]
51. Kuo CC and Bean BP, Slow binding of phenytoin to inactivated sodium channels in rat hippocampal neurons. *Mol Pharmacol*, 1994 46(4): p. 716–25. [PubMed: 7969051]
52. Chen Z, et al., Lidocaine induces a slow inactivated state in rat skeletal muscle sodium channels. *J Physiol*, 2000 524 Pt 1: p. 37–49. [PubMed: 10747182]
53. Carboni M, et al., Slow sodium channel inactivation and use-dependent block modulated by the same domain IV S6 residue. *J Membr Biol*, 2005 207(2): p. 107–17. [PubMed: 16477531]
54. Pavlov E, et al., Block of a prokaryotic voltage-gated sodium channel (NaChBac) by phenytoin and lidocaine. *Biophys J*, 2003 84.
55. Karplus M and McCammon JA, Molecular dynamics simulations of biomolecules. *Nat Struct Biol*, 2002 9(9): p. 646–52. [PubMed: 12198485]
56. Freddolino PL, et al., Molecular dynamics simulations of the complete satellite tobacco mosaic virus. *Structure*, 2006 14(3): p. 437–49. [PubMed: 16531228]
57. Jensen MO, et al., Mechanism of voltage gating in potassium channels. *Science*, 2012 336(6078): p. 229–33. [PubMed: 22499946]
58. Bezanilla F, The voltage sensor in voltage-dependent ion channels. *Physiol Rev*, 2000 80(2): p. 555–92. [PubMed: 10747201]
59. Ragsdale DS, et al., Common molecular determinants of local anesthetic, antiarrhythmic, and anticonvulsant block of voltage-gated Na^+ channels. *Proc Natl Acad Sci U S A*, 1996 93(17): p. 9270–5. [PubMed: 8799190]
60. Yarov-Yarovoy V, et al., Molecular determinants of voltage-dependent gating and binding of pore-blocking drugs in transmembrane segment IIIS6 of the Na^+ channel α subunit. *J Biol Chem*, 2001 276(1): p. 20–7. [PubMed: 11024055]
61. Yarov-Yarovoy V, et al., Role of amino acid residues in transmembrane segments IS6 and IIS6 of the Na^+ channel α subunit in voltage-dependent gating and drug block. *J Biol Chem*, 2002 277(38): p. 35393–401. [PubMed: 12130650]
62. Cestele S and Catterall WA, Molecular mechanisms of neurotoxin action on voltage-gated sodium channels. *Biochimie*, 2000 82(9–10): p. 883–92. [PubMed: 11086218]
63. Vassilev PM, Scheuer T, and Catterall WA, Identification of an intracellular peptide segment involved in sodium channel inactivation. *Science*, 1988 241(4873): p. 1658–61. [PubMed: 2458625]
64. Chiamvimonvat N, et al., Depth asymmetries of the pore-lining segments of the Na^+ channel revealed by cysteine mutagenesis. *Neuron*, 1996 16(5): p. 1037–47. [PubMed: 8630242]

65. Favre I, Moczydlowski E, and Schild L, On the structural basis for ionic selectivity among Na^+ , K^+ , and Ca^{2+} in the voltage-gated sodium channel. *Biophys J*, 1996 71(6): p. 3110–3125. [PubMed: 8968582]
66. Schlief T, et al., Pore properties of rat brain II sodium channels mutated in the selectivity filter domain. *Eur Biophys J*, 1996 25(2): p. 75–91. [PubMed: 9035373]
67. Tsushima RG, Li RA, and Backx PH, Altered ionic selectivity of the sodium channel revealed by cysteine mutations within the pore. *J Gen Physiol*, 1997 109(4): p. 463–475. [PubMed: 9101405]
68. Terlau H, et al., Mapping the site of block by tetrodotoxin and saxitoxin of sodium channel II. *FEBS Lett*, 1991 293(1–2): p. 93–6. [PubMed: 1660007]
69. Penzotti JL, et al., Differences in saxitoxin and tetrodotoxin binding revealed by mutagenesis of the Na^+ channel outer vestibule. *Biophys J*, 1998 75(6): p. 2647–57. [PubMed: 9826589]
70. Jensen MO, et al., Principles of conduction and hydrophobic gating in K^+ channels. *Proc Natl Acad Sci U S A*, 2010 107(13): p. 5833–8. [PubMed: 20231479]
71. Zhu F and Hummer G, Drying transition in the hydrophobic gate of the GLIC channel blocks ion conduction. *Biophys J*, 2012 103(2): p. 219–27. [PubMed: 22853899]
72. Hondeghem LM and Katzung BG, Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. *Biochimica et Biophysica Acta (BBA) - Reviews on Biomembranes*, 1977 472(3–4): p. 373–398. [PubMed: 334262]
73. Hille B, The pH-dependent rate of action of local anesthetics on the node of Ranvier. *J Gen Physiol*, 1977 69(4): p. 475–96. [PubMed: 16078]
74. Tyson JR and Snutch TP, Molecular nature of voltage-gated calcium channels: structure and species comparison. *Wiley Interdisciplinary Reviews: Membrane Transport and Signaling*, 2013 2(5): p. 181–206.
75. Yue L, et al., The cation selectivity filter of the bacterial sodium channel, NaChBac. *J Gen Physiol*, 2002 120(6): p. 845–853. [PubMed: 12451053]
76. Andersen OS, Perspectives on: Ion selectivity. *J Gen Physiol*, 2011 137(5): p. 393–5. [PubMed: 21518827]
77. Qiu H, Shen R, and Guo W, Ion solvation and structural stability in a sodium channel investigated by molecular dynamics calculations. *Biochim Biophys Acta*, 2012 1818(11): p. 2529–35. [PubMed: 22699041]
78. Tang L, et al., Structural basis for Ca^{2+} selectivity of a voltage-gated calcium channel. *Nature*, 2014 505(7481): p. 56–61. [PubMed: 24270805]
79. Li Q, et al., Structural mechanism of voltage-dependent gating in an isolated voltage-sensing domain. *Nat Struct Mol Biol*, 2014 21(3): p. 244–52. [PubMed: 24487958]
80. Stuhmer W, et al., Structural parts involved in activation and inactivation of the sodium channel. *Nature*, 1989 339(6226): p. 597–603. [PubMed: 2543931]
81. Papazian DM, et al., Alteration of voltage-dependence of Shaker potassium channel by mutations in the S4 sequence. *Nature*, 1991 349(6307): p. 305–10. [PubMed: 1846229]
82. Takeshita K, et al., X-ray crystal structure of voltage-gated proton channel. *Nat Struct Mol Biol*, 2014 21(4): p. 352–7. [PubMed: 24584463]
83. Vargas E, Bezanilla F, and Roux B, In search of a consensus model of the resting state of a voltage-sensing domain. *Neuron*, 2011 72(5): p. 713–20. [PubMed: 22153369]
84. Guo J, et al., Structure of the voltage-gated two-pore channel TPC1 from *Arabidopsis thaliana*. *Nature*, 2016 531(7593): p. 196–201. [PubMed: 26689363]
85. Hodgkin AL and Keynes RD, The potassium permeability of a giant nerve fibre. *J Physiol*, 1955 128(1): p. 61–88.
86. Berneche S and Roux B, Energetics of ion conduction through the K^+ channel. *Nature*, 2001 414: p. 73–77. [PubMed: 11689945]
87. Allen TW and Chung SH, Brownian dynamics study of an open-state KcsA potassium channel. *Biochim Biophys Acta*, 2001 1515(2): p. 83–91. [PubMed: 11718664]
88. Corry B, et al., Mechanisms of permeation and selectivity in calcium channels. *Biophys J*, 2001 80(1): p. 195–214. [PubMed: 11159395]

89. Boda D, et al., Ionic selectivity in L-type calcium channels by electrostatics and hard-core repulsion. *J Gen Physiol*, 2009 133(5): p. 497–509. [PubMed: 19398776]
90. Boda D, et al., Combined effect of pore radius and protein dielectric coefficient on the selectivity of a calcium channel. *Phys Rev Lett*, 2007 98(16): p. 168102. [PubMed: 17501467]
91. Yoo J, Wilson J, and Aksimentiev A, Improved model of hydrated calcium ion for molecular dynamics simulations using classical biomolecular force fields. *Biopolymers*, 2016.
92. Neyton J and Miller C, Discrete Ba^{2+} block as a probe of ion occupancy and pore structure in the high-conductance Ca^{2+} -activated K^{+} channel. *J Gen Physiol*, 1988 92(5): p. 569–86. [PubMed: 3235974]
93. Doyle DA, et al., The structure of the potassium channel: molecular basis of K^{+} conduction and selectivity. *Science*, 1998 280(5360): p. 69–77. [PubMed: 9525859]
94. Noskov SY, Berneche S, and Roux B, Control of ion selectivity in potassium channels by electrostatic and dynamic properties of carbonyl ligands. *Nature*, 2004 431(7010): p. 830–4. [PubMed: 15483608]
95. Allen TW, Andersen OS, and Roux B, On the importance of atomic fluctuations, protein flexibility, and solvent in ion permeation. *J Gen Physiol*, 2004 124(6): p. 679–90. [PubMed: 15572347]
96. Eisenman G, Cation selective glass electrodes and their mode of operation. *Biophys J*, 1962 2(2 Pt 2): p. 259–323. [PubMed: 13889686]
97. Bezanilla F and Armstrong CM, Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. *J Gen Physiol*, 1972 60(5): p. 588–608. [PubMed: 4644327]
98. Sun YM, et al., On the structural basis for size-selective permeation of organic cations through the voltage-gated sodium channel. Effect of alanine mutations at the DEKA locus on selectivity, inhibition by Ca^{2+} and H^{+} , and molecular sieving. *J Gen Physiol*, 1997 110(6): p. 693–715. [PubMed: 9382897]
99. Woronzow, Über die Einwirkung des konstanten Stromes auf den mit Wasser, Zuckerlösung, Alkali- und Erdalkalichloridlösungen behandelten Nerven. *Pflug. Arch. ges. Physiol*, 1924 203: p. 300–318.
100. Hodgkin AL and Huxley AF, The dual effect of membrane potential on sodium conductance in the giant axon of Loligo. *J Physiol*, 1952 116(4): p. 497–506. [PubMed: 14946715]
101. Narahashi T, Restoration of action potential by anodal polarization in lobster giant axons. *J Cell Com Physiol*, 1964 64(1): p. 73–96.
102. Adelman WJ Jr. and Palti Y, The effects of external potassium and long duration voltage conditioning on the amplitude of sodium currents in the giant axon of the squid, *Loligo pealei*. *J Gen Physiol*, 1969 54(5): p. 589–606. [PubMed: 5346530]
103. Hoshi T, Zagotta WN, and Aldrich RW, Two types of inactivation in Shaker K^{+} channels: effects of alterations in the carboxy-terminal region. *Neuron*, 1991 7(4): p. 547–56. [PubMed: 1931050]
104. Cannon SC, Brown RH Jr., and Corey DP, A sodium channel defect in hyperkalemic periodic paralysis: potassium-induced failure of inactivation. *Neuron*, 1991 6(4): p. 619–26. [PubMed: 1849724]
105. Cannon SC and Strittmatter SM, Functional expression of sodium channel mutations identified in families with periodic paralysis. *Neuron*, 1993 10(2): p. 317–26. [PubMed: 8382500]
106. Grant AO, Molecular biology of sodium channels and their role in cardiac arrhythmias. *Am J Med*, 2001 110(4): p. 296–305. [PubMed: 11239848]
107. Wang Q, et al., Cardiac sodium channel mutations in patients with long QT syndrome, an inherited cardiac arrhythmia. *Hum Mol Genet*, 1995 4(9): p. 1603–7. [PubMed: 8541846]
108. Lossin C, et al., Epilepsy-associated dysfunction in the voltage-gated neuronal sodium channel SCN1A . *J Neurosci*, 2003 23(36): p. 11289–95. [PubMed: 14672992]
109. Rhodes TH, et al., Noninactivating voltage-gated sodium channels in severe myoclonic epilepsy of infancy. *Proc Natl Acad Sci U S A*, 2004 101(30): p. 11147–52. [PubMed: 15263074]
110. O'Leary ME, Characterization of the isoform-specific differences in the gating of neuronal and muscle sodium channels. *Can J Physiol Pharmacol*, 1998 76(10–11): p. 1041–1050. [PubMed: 10100887]

111. Benz I, et al., Two types of modified cardiac Na⁺ channels after cytosolic interventions at the α -subunit capable of removing Na⁺ inactivation. *Eur Biophys J*, 1997 25(3): p. 189–200. [PubMed: 9037754]
112. West JW, et al., A cluster of hydrophobic amino acid residues required for fast Na⁺ channel inactivation. *Proc Natl Acad Sci U S A*, 1992 89(22): p. 10910–4. [PubMed: 1332060]
113. Kellenberger S, Scheuer T, and Catterall WA, Movement of the Na⁺ Channel Inactivation Gate during Inactivation. *J Biol Chem*, 1996 271(48): p. 30971–30979. [PubMed: 8940085]
114. Rohl CA, et al., Solution structure of the sodium channel inactivation gate. *Biochemistry*, 1999 38(3): p. 855–61. [PubMed: 9893979]
115. McPhee JC, et al., A Critical Role for the S4-S5 Intracellular Loop in Domain IV of the Sodium Channel α -Subunit in Fast Inactivation. *J Biol Chem*, 1998 273(2): p. 1121–1129. [PubMed: 9422778]
116. Hilber K, et al., Interaction between fast and ultra-slow inactivation in the voltage-gated sodium channel. Does the inactivation gate stabilize the channel structure? *J Biol Chem*, 2002 277(40): p. 37105–15. [PubMed: 12138168]
117. Chiu SY, et al., A quantitative description of membrane currents in rabbit myelinated nerve. *J Physiol*, 1979 292: p. 149–66. [PubMed: 314974]
118. Lam K, Wu Z, and Schulten K, Computational Modeling of Sodium Channel Inactivation. *Biophys J*, 2016 110(3): p. 108a.
119. Zeng Z and French C. The Effects of Anti-Epileptic Drug Phenytoin on Slower Processes of Na_v Channels. in *Australian Neurosciences Society 2011 Auckland*.
120. Ellerkmann RK, et al., Slow recovery from inactivation regulates the availability of voltage-dependent Na⁺ channels in hippocampal granule cells, hilar neurons and basket cells. *J Physiol*, 2001 532(2): p. 385–397. [PubMed: 11306658]
121. Ulbricht W, Sodium channel inactivation: molecular determinants and modulation. *Physiol Rev*, 2005 85(4): p. 1271–301. [PubMed: 16183913]
122. Balser JR, et al., External pore residue mediates slow inactivation in μ 1 rat skeletal muscle sodium channels. *J Physiol*, 1996 494 (Pt 2): p. 431–42. [PubMed: 8842002]
123. Tomaselli GF, et al., A mutation in the pore of the sodium channel alters gating. *Biophys J*, 1995 68(5): p. 1814–27. [PubMed: 7612823]
124. Kambouris NG, et al., Mechanistic link between lidocaine block and inactivation probed by outer pore mutations in the rat micro1 skeletal muscle sodium channel. *J Physiol*, 1998 512 (Pt 3): p. 693–705. [PubMed: 9769414]
125. Zhang Z, et al., A negatively charged residue in the outer mouth of rat sodium channel determines the gating kinetics of the channel. *Am J Physiol Cell Physiol*, 2003 284(5): p. C1247–54. [PubMed: 12540378]
126. Ong B-H, Tomaselli GF, and Balser JR, A Structural Rearrangement in the Sodium Channel Pore Linked to Slow Inactivation and Use Dependence. *J Gen Physiol*, 2000 116(5): p. 653–662. [PubMed: 11055994]
127. Wang SY and Wang GK, A mutation in segment I-S6 alters slow inactivation of sodium channels. *Biophys J*, 1997 72(4): p. 1633–40. [PubMed: 9083667]
128. O'Reilly JP, Wang SY, and Wang GK, Residue-specific effects on slow inactivation at V787 in D2-S6 of Na_v1.4 sodium channels. *Biophys J*, 2001 81(4): p. 2100–2111. [PubMed: 11566781]
129. Vedantham V and Cannon SC, Rapid and slow voltage-dependent conformational changes in segment IVS6 of voltage-gated Na⁺ channels. *Biophys J*, 2000 78(6): p. 2943–58. [PubMed: 10827974]
130. Zarrabi T, et al., A molecular switch between the outer and the inner vestibules of the voltage-gated Na⁺ channel. *J Biol Chem*, 2010 285(50): p. 39458–70. [PubMed: 20926383]
131. McGregor MJ, Islam SA, and Sternberg MJ, Analysis of the relationship between side-chain conformation and secondary structure in globular proteins. *J Mol Biol*, 1987 198(2): p. 295–310. [PubMed: 3430610]
132. Ballesteros JA, et al., Serine and Threonine Residues Bend α -Helices in the $\chi_1=g^-$ Conformation. *Biophys J*, 2000 79(5): p. 2754–2760. [PubMed: 11053148]

133. Deupi X, et al., Ser and Thr residues modulate the conformation of Pro-kinked transmembrane α -helices. *Biophys J*, 2004 86(1 Pt 1): p. 105–15. [PubMed: 14695254]
134. Zhao Y, Scheuer T, and Catterall WA, Reversed voltage-dependent gating of a bacterial sodium channel with proline substitutions in the S6 transmembrane segment. *Proc Natl Acad Sci USA*, 2004 101(51): p. 17873–8. [PubMed: 15583130]
135. Catterall WA, Ion channel voltage sensors: structure, function, and pathophysiology. *Neuron*, 2010 67(6): p. 915–28. [PubMed: 20869590]
136. George AL Jr., Inherited disorders of voltage-gated sodium channels. *J Clin Invest*, 2005 115(8): p. 1990–9. [PubMed: 16075039]
137. England S and de Groot MJ, Subtype-selective targeting of voltage-gated sodium channels. *Br J Pharmacol*, 2009 158(6): p. 1413–25. [PubMed: 19845672]
138. Tarnawa I, Bolcskei H, and Kocsis P, Blockers of voltage-gated sodium channels for the treatment of central nervous system diseases. *Recent Pat CNS Drug Discov*, 2007 2(1): p. 57–78. [PubMed: 18221218]
139. Mike A and Lukacs P, The enigmatic drug binding site for sodium channel inhibitors. *Curr Mol Pharmacol*, 2010 3(3): p. 129–44. [PubMed: 20565383]
140. Bean BP, Cohen CJ, and Tsien RW, Lidocaine block of cardiac sodium channels. *J Gen Physiol*, 1983 81(5): p. 613–42. [PubMed: 6306139]
141. Wang GK, et al., Inhibition of sodium currents by local anesthetics in chloramine-T-treated squid axons. The role of channel activation. *J Gen Physiol*, 1987 89(4): p. 645–67. [PubMed: 2438374]
142. Cahalan MD, Local anesthetic block of sodium channels in normal and pronase-treated squid giant axons. *Biophys J*, 1978 23(2): p. 285–311. [PubMed: 687766]
143. Balser JR, et al., Local anesthetics as effectors of allosteric gating. Lidocaine effects on inactivation-deficient rat skeletal muscle Na channels. *J Clin Invest*, 1996 98(12): p. 2874–86. [PubMed: 8981936]
144. Bennett PB, et al., On the molecular nature of the lidocaine receptor of cardiac Na^+ channels. Modification of block by alterations in the α -subunit III-IV interdomain. *Circ Res*, 1995 77(3): p. 584–92. [PubMed: 7641328]
145. Balser JR, et al., Functional consequences of lidocaine binding to slow-inactivated sodium channels. *J Gen Physiol*, 1996 107(5): p. 643–58. [PubMed: 8740377]
146. Sheets PL, Jarecki BW, and Cummins TR, Lidocaine reduces the transition to slow inactivation in $\text{Na}_v1.7$ voltage-gated sodium channels. *Br J Pharmacol*, 2011 164(2b): p. 719–30. [PubMed: 21232038]
147. Sheets PL, et al., Differential block of sensory neuronal voltage-gated sodium channels by lacosamide [(2R)-2-(acetylamino)-N-benzyl-3-methoxypropanamide], lidocaine, and carbamazepine. *J Pharmacol Exp Ther*, 2008 326(1): p. 89–99. [PubMed: 18378801]
148. Hebeisen S, et al., Eslicarbazepine and the enhancement of slow inactivation of voltage-gated sodium channels: a comparison with carbamazepine, oxcarbazepine and lacosamide. *Neuropharmacology*, 2015 89: p. 122–35.
149. Sheets MF, et al., Sodium channel molecular conformations and antiarrhythmic drug affinity. *Trends Cardiovasc Med*, 2010 20(1): p. 16–21. [PubMed: 20685573]
150. Fozzard HA, Sheets MF, and Hanck DA, The sodium channel as a target for local anesthetic drugs. *Front Pharmacol*, 2011 2: p. 68. [PubMed: 22053156]
151. McNulty MM, et al., Charge at the lidocaine binding site residue Phe-1759 affects permeation in human cardiac voltage-gated sodium channels. *J Physiol*, 2007 581(Pt 2): p. 741–55. [PubMed: 17363383]
152. Wang GK, Quan C, and Wang S, A common local anesthetic receptor for benzocaine and etidocaine in voltage-gated $\text{mu}1 \text{Na}^+$ channels. *Pflugers Arch*, 1998 435(2): p. 293–302. [PubMed: 9382945]
153. Qu Y, et al., Molecular determinants of drug access to the receptor site for antiarrhythmic drugs in the cardiac Na^+ channel. *Proc Natl Acad Sci U S A*, 1995 92(25): p. 11839–43. [PubMed: 8524860]
154. Ragsdale DS, et al., Molecular determinants of state-dependent block of Na^+ channels by local anesthetics. *Science*, 1994 265(5179): p. 1724–8. [PubMed: 8085162]

155. Raju SG, et al., Exploring volatile general anesthetic binding to a closed membrane-bound bacterial voltage-gated sodium channel via computation. PLoS Comput Biol, 2013 9(6): p. e1003090. [PubMed: 23785267]
156. Quandt FN, Modification of slow inactivation of single sodium channels by phenytoin in neuroblastoma cells. Mol Pharmacol, 1988 34(4): p. 557–65. [PubMed: 2845252]
157. Muroi Y and Chanda B, Local anesthetics disrupt energetic coupling between the voltage-sensing segments of a sodium channel. J Gen Physiol, 2009 133(1): p. 1–15. [PubMed: 19088384]
158. Li P, et al., The gating charge pathway of an epilepsy-associated potassium channel accommodates chemical ligands. Cell Res, 2013 23(9): p. 1106–18. [PubMed: 23797855]
159. Smart OS, Neduvilil JG, Wang X, Wallace BA, Sansom MSP, HOLE: A program for the analysis of the pore dimensions of ion channel structural models. J. Molecular. Graphics, 1996 14(6): p. 354–360.

Figure 1:

Comparing Na_v and BacNa_v channels. (top) Arrangement of the transmembrane α subunit of a mammalian Na_v channel showing the four-domain (I to IV) structure with six α -helical transmembrane segments in each domain. Segments S1 to S4 form the voltage sensing domain. The positively charged residues of gating segments S4 are highlighted in purple. The S5 and S6 segments and the pore loop region between them form the pore domain. The residues forming the selectivity locus DEKA are highlighted as colored circles, in green, red blue and orange respectively. (bottom) Arrangement of the transmembrane α subunit of a bacterial Na_v channel, showing the similarity to one domain of the Na_v channel α subunit.

Figure 2:

The structure of channel Na_vAb. Main structural elements of channel Na_vAb showing the arrangement of segments S1 to S6 forming the pore domain and the voltage-sensing domains. For clarity, the segments forming the pore domain of one subunit and the voltage sensing domain of another are omitted. A more detailed representation of the selectivity filter is added as an insert (colored sticks, C in cyan, N in blue and O in red, 2 subunits shown), displaying its specific sequence of residues TLES and the 3 binding sites for Na⁺ S_{HFS}, S_{CEN} and S_{IN} as red dashed circles. At the level of the voltage sensing domain, the key residues playing a role in gating are represented as colored sticks and labelled. Additionally, the residues forming the gating hinge, the intracellular negative-charge cluster (INC), the extracellular negative-charge cluster (ENC) and the hydrophobic constriction site (HCS) are also indicated. One of the the openings between the membrane and the pore (fenestrations) formed by adjacents segments S6 is circled in black dashes.

Figure 3:

Sequence alignment of the selectivity filter of bacterial Na_v channels NaChBac, Na_vAb, Na_vMs and Na_vRh, and mammalian Na_v1.2 and Ca_v1.2 channels. Negatively charged residues are colored in red, positively charged residues in blue, aromatic residues in orange and polar residues in green.

Figure 4:

The conduction process of sodium in Na_vAb relies on knock-on and pass-by processes in the presence of 3 ions. 2D free energy projections for Na⁺ ions in Na_vAb, when three ions occupy the selectivity filter, with z_1 , z_2 and z_3 corresponding to the positions of the bottom (1), middle (2), and top (3) ions, and z_{23} the centre of mass of the upper ions (2 and 3). Contouring is at 0.5 kcal/mol, with lowest free energy pathways as white dashed curves. The permeation mechanism is shown, with free energies relative to state A in kcal/mol ($\sim \pm 0.5$ kcal/mol). Adapted from [31].

Figure 5:

Gating in BacNa_v channel pore domain. Comparison of the pore domain of published X-ray structures of bacterial Na_v channels. Plot of pore radius versus position along the *z*-axis for Na_vAb (pdb 3rvy, green), Na_vRh (pdb 4dwx, blue), Na_vMs(1) (pdb 4f4l, orange), Na_vMs(2) (pdb 4cbc, red) and Na_vAe (pdb 4lto, violet) and backbone superimposition of the same channels using ribbons with the same color code, with Na_vAb pore volume represented as a grey mesh. For clarity, only 2 subunits of the pore domain are represented. Pore volume and radii were calculated with Hole [159].

Figure 6:

Slow inactivation in mammalian Na_v channels. Schematic representation of a mammalian Na_v channel and the residues known to modulate slow inactivation: DI-selectivity filter W402 [122–124] (green dot), DI-selectivity filter E403 [125] (red dot), DIII-selectivity filter F1236 [126] (blue dot), DI-S6 N434 [127] (orange dot), DII-S6 V787 [128] (purple dot), DIV-S6 F1764 [53] (pink dot), DIV-S6 I1575 and DIV-S6 V1583 [129] (light blue dot). The numbering corresponds to the rat Na_v1.4 channel sequence unless indicated otherwise.

Figure 7: Superimposed bottom views of the proposed inactivated asymmetric conformation described by Payandeh et al. [16] (PDB:4EKW segments AB, yellow and orange cylinders) and the conformation obtained after a 2.5 μ s of Na_vAb (blue and purple cylinders), showing the displacement of the helices S6. The black dashed line highlights the asymmetrically collapsed gate, being similar in both structures.

Figure 8:
Conformational changes relevant to slow inactivation in the pore domain of Na_vAb. Segment S6 (yellow cylinder) bends near residue F203 (red sticks), breaking the bond between residues I202 and T206 (colored sticks), and induces a tilt of the top pore helix, P2 (light blue cylinder), and an upward displacement of a selectivity filter subunit, leading to a collapse of the filter.

Figure 9:

Binding sites and access pathways revealed by MD simulation for benzocaine and phenytoin in Na_vAb . For clarity only 2 subunits are shown for the voltage sensing domain and 3 for the pore domain. Residues important for binding are shown as colored sticks (F203 in red, N211 in dark blue and S4 R102, R105 and R108 in yellow). Horizontal black lines mark the limit of the lipid bilayer while black arrows show simplified access pathways to specific binding sites for the drugs specified. Benzocaine (green) and phenytoin (blue) are shown as stick.